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Studying mechanisms of transcranial brain stimulation: a combined TMS–tES study

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Aivojen toiminta perustuu pitkälti sähköisiin viesteihin. Aivoihin voidaan luoda keinotekoisesti toimintaa kallon ulkopuolelta sähkömagneettisen stimulaation avulla. Tällaisia stimulaatiomenetelmiä ovat muun muassa transkraniaalinen magneettistimulaatio (TMS), tasavirtastimulaatio (tDCS) sekä vaihtovirtastimulaatio (tACS). Menetelmien tiedetään vaikuttavan aivojen sähköiseen viestintään, mutta vaikutusmekanismit eivät ole täysin selviä. Tämän työn tarkoituksena on ymmärtää sähkömagneettisen stimulaation vaikutusmekanisemeja entistä paremmin.

Selvittääksemme sähkömagneettisen stimulaation vaikutusmekanismeja tässä työssä tehtiin sarja mittauksia, joissa TMS yhdistettiin samanaikaiseen sähköstimulaatioon. Erityisesti tässä työssä tutkittiin, kuinka sähköstimulaation aivoihin tuottaman virran suunta vaikuttaa TMS:n herättämiin lihasvasteisiin, joita mitattiin lihassähkökäyrällä (EMG) sormesta.

Tehtyjen mittausten perusteella ei voida vielä tehdä selkeitä johtopäätöksiä vaikutusmekanismeistä. Sähköstimulaation aiheuttamalla virran suunnalla ei havaittu olevan selkeää vaikutusta TMS:n aiheuttamiin lihasherätevasteisiin. Tämä voi osittain johtua tACS:n sekä tDCS:n heikosta stimulaatiotehosta, milloin sähköstimulaation aiheuttamat reaktiivisuusmuutokset eivät erotu mittausepätarkkuuksien alta. Toisaalta tulokset voivat myös viitata siihen, että sähkömagneettisen aivostimulaation vaikutusmekanismeja ei vielä ymmärretä tarkasti.

Avainsanat: Transkraniaalinen magneettistimulaatio, Lihasvasteet, Lihassähkökäyrä, Transkraniaalinen tasavirtastimulaatio, Transkraniaalinen vaihtovirtastimulaatio

AALTO UNIVERSITY
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ABSTRACT OF THE
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Most of the communication in the brain is based on electric charges. With electromagnetic stimulation, we can disturb the normal state of the brain and generate artificial brain activity. Transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES) are commonly used stimulation methods. We know that these methods affect the neural communication, but their working mechanisms are not perfectly clear. With this thesis, we try to gain better understanding of the underlying mechanisms.

To understand the underlying mechanisms, we combined two stimulation methods, TMS and tES. In particular, we were interested in how the direction of the tES-induced electric field affected the TMS-evoked muscle responses that were measured from the target muscle, using electromyography (EMG).

The obtained results did not provide clear evidence to support the current understanding of the working mechanisms of the electromagnetic stimulation. We could not find an effect of the tES current direction on the MEP responses. It is possible that the tES-stimulation strength is too low to noticeably affect the EMG responses, given measurement inaccuracies. On the other hand, the results may indicate that the underlying mechanisms of electromagnetic stimulation are still not perfectly understood

Keywords: Transcranial direct current stimulation, Electromyography
Transcranial alternating current stimulation,
Transcranial magnetic stimulation

Preface

I want to thank my instructor Tuomas Mutanen for all the patience he had and the useful comments he gave always very quickly. I'm sure the process taught us both a lot. Without him the quality of this work would not be even close to what it is now. I also want to thank my supervisor Risto Ilmoniemi for understanding and useful advice.

Writing this thesis took longer than anyone expected as the project began already in 2014. The project, and life, was full of ups and downs before we got to the end.

Espoo, 31.10.2016

Matleena M. M. Kukkonen

Contents

List of abbreviations

ABP	Abductor pollicis brevis
AC	Alternating current
DC	Direct current
EEG	Electroencephalography
EMG	Electromyography
FDI	First dorsal interosseus
MA	Moving average
MRI	Magnetic resonance imaging
MT	Motor threshold
M1	Primary motor cortex
nTMS	Navigated transcranial magnetic stimulation
SEM	Standard error of the mean
tACS	Transcranial alternating current stimulation
tDCS	Transcranial direct current stimulation
tES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation

1 Introduction

Information transmission in the brain is largely based on electric charges. Everything we do, think or sense is caused by (or leads to) brain cells (neurons) sending messages to each other through action potentials. These messages can also be caused and affected artificially with electromagnetic stimulation.

One way to elicit artificial messages in the brain is transcranial magnetic stimulation (TMS). It is a method for stimulating the superficial parts of the brain, without opening the skull, with electromagnetism. In TMS, one creates a quick strong magnetic pulse that induces an electric field inside the brain. The electric field can activate the targeted brain cells, and through inter-neural signalling other neuronal populations can also be activated. The evoked cortical activity can also send signals to a distant target part of the body. For example, TMS applied to the main area responsible for motor activity (primary motor cortex M1) can cause involuntary activations of the target muscle. This activation of the target muscle can be measured with electromyography (EMG).

The neuronal messaging can be modulated by using transcranial electrical stimulation (tES). In tES, usually weak electrical currents are delivered to the head via electrodes attached to the scalp. Some part of the injected current travels through the skull to the brain. The currents used in electrical stimulation can have direct (tDCS) or alternating current (tACS) waveforms. The applied currents are typically not strong enough to create as strong electric fields in the brain as TMS pulses. Thus, with usual intensities, tES is not believed to produce neuronal messages by itself, but enhance or inhibit their occurrence. It is thought that the working mechanism of tES might be that it affects the resting membrane potential of the neurons in the brain [1]. This way, it makes the cells less or more likely to produce neuronal messages, *i.e.*, to fire action potentials. The working mechanism, however, is not perfectly clear.

In this thesis, we are combining TMS and tES to obtain better understanding of the underlying mechanisms of these methods. We compare the effects of opposite current directions of tES by simultaneously stimulating the brain with TMS. Our aim is to study weather the tES current has any effect on the EMG response of the target muscle, caused by the TMS, in particular we studied, whether the direction of the tES current plays a significant role in the measured MEPs. To quantify the effects of tES on muscle responses of TMS, we study the number and amplitudes of clear muscle responses.

As a result of the experiments, we expect to see a difference between the negative and positive electric fields caused by tES stimulation. The opposite current directions should affect the TMS-evoked muscle responses in an opposite way. For example, a tES current parallel with the TMS-induced electric field can be expected to enhance the effects of TMS, and vice versa. Similarly, the value of the alternating current at TMS stimulation time should correlate with the stimulation response. The results obtained from this work provide us with further evidence of the mechanisms behind the suggested simulation methods.

2 Background

A human brain consists of approximately 100 billion neurons, a large number of glia cells, and other supporting structures, such as blood vessels. Brain cells are specialized in transferring and storaging data. Each neuron consists of a cell body (soma), dendrites and axon. [2]

Dendrites receive information from other cells. The received information can be either excitatory or inhibitory. Some information can also arrive straight at soma. In the soma, the signals arriving from various cells are summed up. If the net input of information exceeds the threshold value, the cell sends a message forwards along the axon, resulting in an action potential. [2]

The signals in neurons are chemical or electrical. The electrical signals are based on a potential difference over the cellular membrane that is due to different ion concentration inside and outside the cell. The concentration difference of mainly potassium, sodium and chlorine ions is maintained actively with ion pumps. In non-active cells, this potential difference is called the resting membrane potential. If the membrane voltage is depolarized enough from the resting membrane potential, the cell fires an action potential. [2]

At the axon end, the information is transferred from a cell to another. The transfer of information happens through synapses that are located in the end of the axon. When the action potential reaches the end of the axon, it causes synapses to release neurotransmitters that diffuse to neighbouring cells. Axon ends may have many synapses connected to several different dendrites, somas or target muscles. [2]

The outermost layer of the brain is called the cortex. It is a wrinkled surface consisting of fissures (sulci) and ridges (gyri). The two primary types of cortical neurons are pyramidal cells and interneurons. Pyramidal cells are usually excitatory and oriented perpendicular to the cortical surface while inhibitory interneurons can be oriented in all directions. [2]

The cortex is divided into several functional areas, such as, motor cortex, somatosensory cortex and visual areas. The areas are usually located in specific part of gyri or sulci with only little interpersonal variation. [2] Cognitive tasks often require interaction between multiple functional areas. Communication requires high connectivity between brain areas. One possible way is that the communication is mediated by synchronization [3].

Post-synaptic currents caused by multiple action potentials fired by a single neuron or a small group of neurons can be measured with electroencephalography (EEG). A perdiodic variation seen in measurements is called neural oscillation. The oscillation frequency can vary between 1 an 600 Hz, and is often categorized as different frequency bands: delta 1–3 Hz, theta 4–7 Hz, alpha 8–13 Hz, gamma 30–80 Hz, fast 80–200 Hz, ultra fast 200–600 Hz. Synchronization and desynchronization of these oscillations in different parts of the brain is a likely mechanism for neural communication. [3]

2.1 History of electromagnetic stimulation

In the late 18th century, Luigi Galvani discovered that nerves and muscles of a frog are electrically excitable by stimulating them with electricity with the coarse methods he had in his use [4]. This was the beginning of electrical stimulation. The first one to test if the brain was electrically excitable was Galvani's nephew Aldini [4]. In the turn of the 19th century, he stimulated the heads and limbs of dead subjects and observed the muscles reacting. He was not, however, actually stimulating the brain like he thought, but the muscles.

In the early 19th century, better stimulation techniques were discovered [4]. Techniques with a finer control over duration, intensity and area of stimulation were developed. Also electromagnetic induction, that TMS is based on, was discovered by Faraday in 1831. These methods made it possible to go forwards with brain stimulation studies.

In 1870, two German physiologists Fritsch and Hitzig [5] did a pioneering mapping study of the cortex with animals. They discovered that stimulating different locations of the brain made different parts of the body move. In 1874, Bartholow [6] described the stimulation of exposed human cerebral cortex with electric currents eliciting movements in the opposite side of the body.

The first magnetic nerve stimulation in a frog was reported by Kolin *et al.* in 1959 [7] and only few years later in 1965, peripheral nerve of a human was stimulated by Bickford and Fremming [8]. They used, however, oscillatory magnetic field that lasted 40 ms and the long stimulus made it impossible to record a muscle response. It took few decades before Polson, Barker and Freeston [9] recorded the first motor-evoked potentials (MEPs) obtained by median nerve magnetic stimulation in 1982. Only a few years later in 1985 the same group succeeded in stimulating brain tissue with transcranial magnetic stimulation (TMS) [10]. Since then, TMS has been an important tool for investigating motor pathways.

The rise of TMS led to the revitalization of tDCS. In 1962, Creutzfeld *et al.* [11] had already showed with cats that the spontaneous neuronal firing rates, during anodal and cathodal tDCS stimulating were increasing or reducing respectively. In 2000 it was demonstrated that tDCS induces cortical exitability changes in the human cortex as well [12].

2.2 Transcranial magnetic stimulation

The resting potential in neurons can be disturbed artificially with transcranial magnetic stimulation. In TMS, a strong electric current pulse is driven through a coil, which generates a changing magnetic field. [13] Current generates a magnetic field according to the Biot–Savart law:

$$\mathbf{B}(\mathbf{r},t) = \frac{\mu_0}{4\pi} I(t) \oint_C \frac{d\mathbf{l}(\mathbf{r}') \times (\mathbf{r}-\mathbf{r}')}{\mid \mathbf{r}-\mathbf{r}' \mid^3}\,, \tag{1}$$

where $\mathbf{B}(\mathbf{r},t)$ is the strength of magnetic field in location $\mathbf{r}$, $d\mathbf{l}(\mathbf{r}')$ is a differential element of a wire and μ_0 is the permeability of vacuum. The changing magnetic

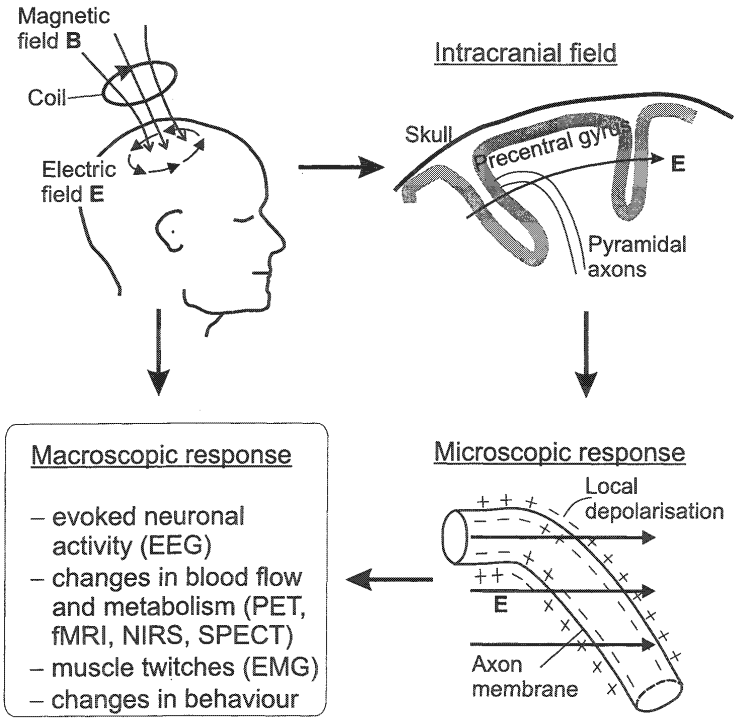

Figure 1: Current understanding of the TMS working principle. Magnetic field $\mathbf{B}$, generated by the quick current in the coil induces an electric field $\mathbf{E}$ in the brain. Electric field sets free charges to motion in the brain and when this motion is interrupted local depolarisation occurs. The direction of electric field should be roughly perpendicular to the axon bends of the cells one wants to activate (Figure from [13]).

field, on the other hand, induces an electric field $\mathbf{E}$ in the brain, according to the Faraday law:

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}\,. \tag{2}$$

The induced electric field sets free charges to motion inside the brain. At the cellular level, the charges try to go the along low-resistant pathways. When the free motion caused by TMS is interrupted by the membrane, the membrane potential is altered and cells tend to depolarize or hyperpolarize. This is why axon bends or synaptic terminals in axon ends are most likely excited (Fig. 1). The strength of the electric field in the brain needed to elicit this neuronal activation is of order 100 mV/mm. When the conductivity of the brain is about 0.4 S/m, the cortical current density is then about 40 μA/mm^2. [13]

To better understand the polarization of neurons, a simplified physical model can be used. Potential V of an isolated long axon at subthreshold can be described

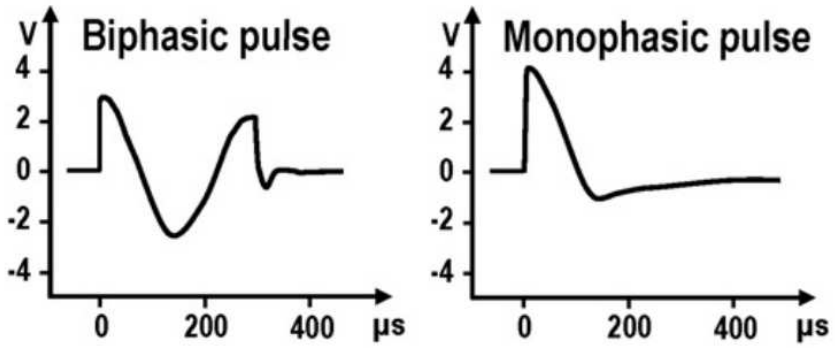

Figure 2: Biphasic and monophasic pulses differ in the pulse shape and duration. The figures show the pulse shape at the distance of 1.5 cm from a figure-of-eight coil. Modified from [18].

by the cable equation:

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} - V - \tau \frac{\partial V}{\partial t} = f(x,t) = \lambda^2 \frac{\partial E_x}{\partial x}\,, \tag{3}$$

where $\lambda = \sqrt{\frac{r_m}{r_i}}$ and $\tau = c_m r_m$. c_m and r_m are the membrane capacitance and resistance per unit length, respectively, and r_i is the axoplasm resistance per unit length. E_x is the x component of electric field $\mathbf{E}$. [14]

From the cable equation, we can see that in a homogenous medium, long axons will be depolarized when the $\frac{\partial E_x}{\partial x}$ is negative and hyperpolarized when it is positive and the gradient value is sufficiently high. The largest gradient is usually found at axon ends and bends. To calculate the activation function f, one needs to know the coil shape, location and characteristics of the surrounding tissue [15]. Cable equation is a simplified model and thus it does not explain the effects of more complicated geometric shapes. For example, when stimulating short axons or close to the axon end, the field transverse to the axon may significantly affect the activation [16]. However, cable equation is useful for the theoretical analysis of TMS.

The waveform and current direction have to be also taken into account when designing a TMS experiment. The most common current pulse forms passing through the stimulation coil are monophasic and biphasic. Both current forms induce biphasic voltage in the brain, because the sign of magnetic field changes when electric current starts to return to zero from the peak value. Only biphasic coils create sufficiently large electric fields in the second phase of the pulse to stimulate twice. The current patterns are shown in Fig. 2. Due to current patterns biphasic coils produce more complex pattern of cortical activation than monophasic coils [17]. It is possible that different interneurons are stimulated with the second stimulating part with biphasic coils.

Coil form affects the distribution of the local magnetic field. Figure-of-eight coils are the most commonly used coils. They consist of two adjacent ring coils. Figure-

of-eight coils produce a focal magnetic field with well-defined directionality at the target point (Fig. 3).

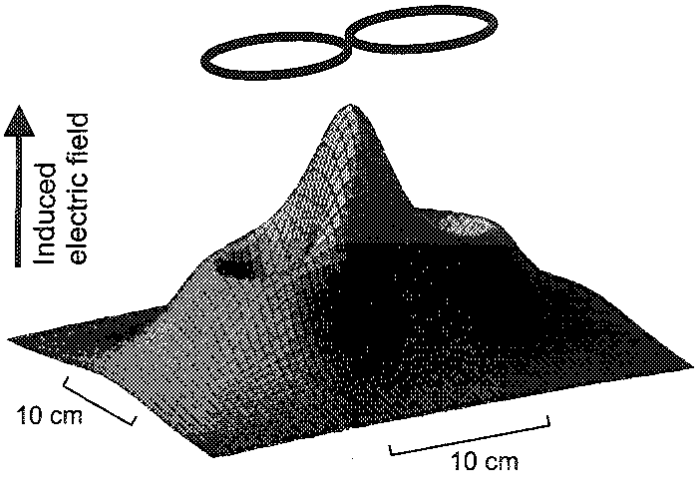

Figure 3: The electric field induced by a figure-of-eight coil. Modified from [13].

A modern neuronavigation system utilizes the subject-specific magnetic resonance imaging (MRI), allowing much more accurate stimulation targeting than in the earlier studies [19, 20]. Navigation allows real-time tracking of the TMS coil over the individual MRI reconstruction of the head of the subject. The MRI reconstruction can be aligned with subject's head to allow accurate stimulation to the specific region of interest in the brain. [21] In the stimulation of the motor cortex, the coil is usually oriented along the posterior-anterior direction, perpendicular to the central sulcus.

2.3 Transcranial electrical stimulation

Transcranial electrical stimulation (tES) covers all non invasive brain stimulation techniques that deliver electrical current directly to the scalp. These techniques include transcranial direct currents stimulation (tDCS) and transcranial alternating current stimulation (tACS). In tES, two, or less commonly more, electrodes are placed on the scalp. Current is applied between the electrodes, some of the current passing through the skull to the brain.

In tDCS, a direct electric current is applied on the electrodes attached to the scalp producing an electric field in the brain. Unlike in TMS, currents produced by tDCS are not sufficient to cause action potentials directly. Instead, tDCS is believed to modulate the brain activity. In tDCS, a low, typically 0.5–2-mA current, is driven from one electrode called the cathode, to another electrode, called the anode. The current of 2 mA driven by a tDCS machine produces currents in the cortex of roughly

0.1 A/m^2, corresponding to an electric field of 0.22 V/m [22]. This means that only a small proportion of the applied tDCS current penetrates through the skin and the insulating skull.

The stimulation effects can be altered by changing the driven currents in the brain. Important parameters are the polarity and the amplitude of the current. [12] The locations of the electrodes can also change the electric field in the brain and the effects are shown to depend on both the reference and the stimulating electrodes' locations [23]. The size and orientation of the electrodes affect the electric field as well [24].

The research field widely accepts two mechanisms that explain the tDCS-elicited modulation in the brain activity. The short-term effects are believed to be based on tDCS modulating the resting membrane potential to either hypo- or hyperpolarized state. Long term effects are thougth to take place due to synaptic modulation causing long term potentiation or long term depression. [25]

The tDCS effects, however, are highly variable between different individual subjects [26]. This may be due to each subject having a unique brain geometry and thus a need for a different electric field direction and magnitude to enable polarizing the electric field. The estimation of the tES evoked electric field inside the brain is a complex task, because the current passes through the scalp, skull, meninges and cerebrospinal fluid before the brain. Unfortunately, the exact conductive properties of different tissues are not well known. [27] Also, the electric field is not evenly spread on the cortex, but can have several local maxima under and between the electrodes. [27, 23, 28] An example of an electric field computation can be seen in Fig. 4. Using multiple small electrodes can help at targeting the stimulation better, at least when simulating tES-current flow in the individual anatomy [29].

In many studies, large 25–35 cm^2 sponge electrodes moistened with NaCl solution are used. Contacts between the electrodes and the scalp can also be made with electrode cream instead of NaCl solution. In a typical study, tDCS is used long periods at the time, and stimulation of 5–20 minutes is common. [30]

Transcranial alternating current stimulation (tACS) is an electrical stimulation form where the current does not stay constant. Instead, the used wave form is sinusoidal. There are more parameters to change in tACS than in tDCS. In addition to the stimulation length, intensity and electrode placing, for tACS, also the frequency, wave shape and phase of the stimulation have to be specified. Frequencies can vary from close to DC to several hundred kHz. [31]

Transcranial alternating current stimulation is thought to affect the cortical oscillations by synchronizing or desynchronizing the neuronal populations [31]. The effect probably depends on the frequency of the stimulation. Low frequencies, such as, 1kHz, probably are not sufficient to interfere with oscillations, but affects the biochemical mechanisms leading to short-term plasticity [31].

2.4 Response to stimulation on motor cortex

Motor cortex stimulation can induce D-waves and I-waves in the pyramidal tract. D-wave is a tripolar wave complex and I-waves are smaller voltage deflections [32].

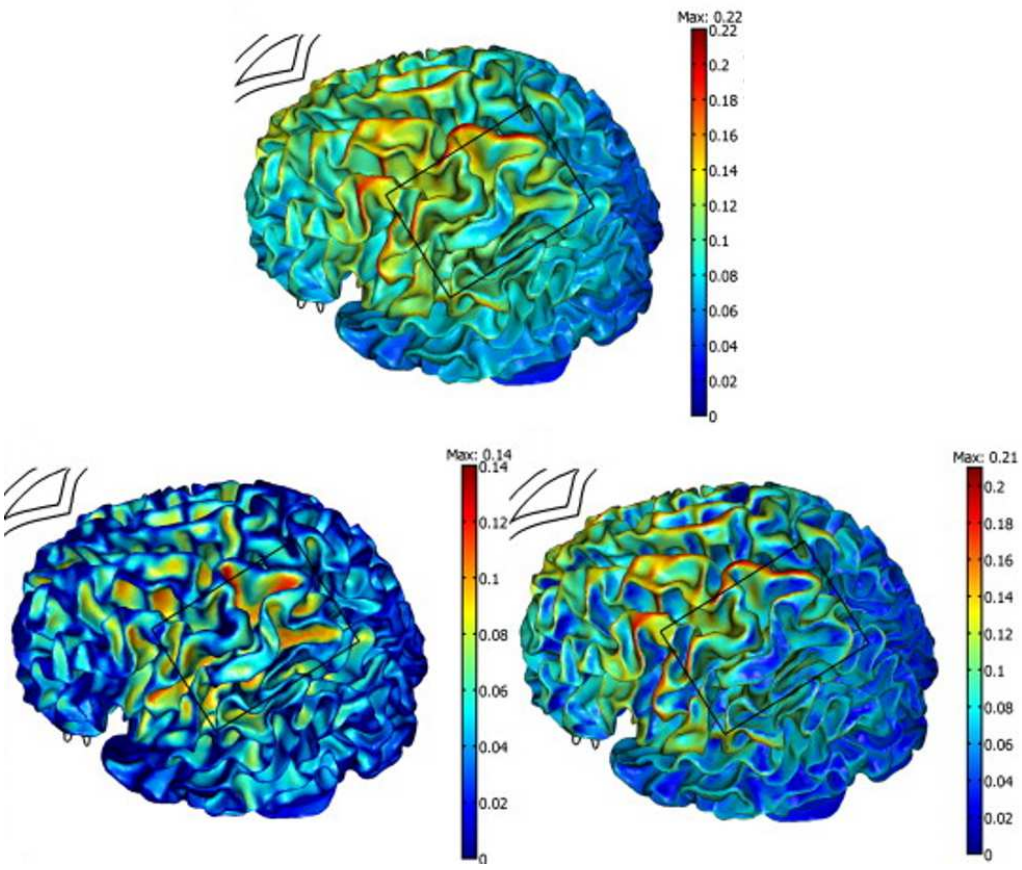

Figure 4: Electric field distributions induced by tES on the white matter surface of the brain. [28]

It is thought that D-waves result from the direct activation of the corticospinal tract and I-waves are the result of trans-synaptic activation of pyramidal cells [17, 32].

Stimulation of the motor cortex can cause activation of small hand muscles at around 25 ms after stimulation. [33] The reactions of the small muscles in the hand caused by the exitation of corticospinal tract can be measured noninvasively using electromyography (EMG). EMG records electrical activity produced by the target skeletal muscles. The responses seen in the EMG are called the motor evoked potentials (MEP).

Motor evoked potentials can be easily modulated if tasks, such as, movement preparation [34], observation [35] as well as other non-target muscle control are done simultaneously with stimulation [36]. MEP amplitudes are highly variable even with the same subject and identical stimuli. [37] (Fig. 5).

It is thought that TMS-stimulation activates the pyramidal cells of the motor tract mainly indirectly, but sometimes also directly [38]. Relatively strong tES on the other hand might excite neurons directly producing direct D-waves. This is implied by how the response latency to the TMS stimulus in the contracting muscle was 1.4—2.7 ms slower in comparison with the electrical stimulus [33]. The direction

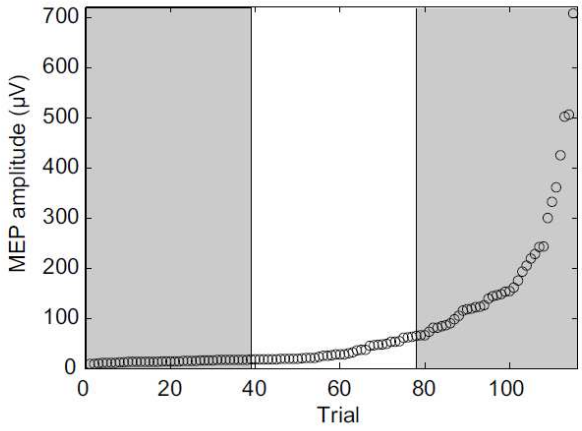

Figure 5: MEP amplitudes measured with identical parameters. The MEP amplitudes are a highly variable parameter. The 1/3 largest and 1/3 smallest amplitudes of the trial are shaded.[37].

and intensity of TMS-stimulation also affect the resulting waves [39, 38].

The amplitude modulation of MEPs has been reported to be one of the after effects of TES. According to a review, MEP-amplitude modulation is the only clear effect generated by tDCS [40]. MEP-amplitude modulation is also a reported after effect of tACS [41]. Antal et al. delivered 1-Hz AC stimulation to the primary motor cortex and showed a trend towards MEP inhibition, when measured after the tACS stimulation [41]. It has been found that the 140-Hz AC stimulation causes the largest MEP increases after stimulation when compared with 80-Hz and 250-Hz stimulation [42]. 80-Hz did not increase MEP amplitudes significantly at any point from 0 to 60 minutes after stimulation. Similar results in the modulation of amplitudes generated by tACS stimulation can be found in the literature (for example [43, 44, 45]).

2.5 Combined TMS–TDCS studies

There are several studies where TMS has been combined with tDCS or tACS to better understand how tES works. In most studies, however, the emphasis has been on measuring the after effects and not the immediate effects during electrical stimulation. One of the few simultaneous stimulation studies discovered tDCS-elicited exitability changes in the motor cortex, quantifying the excitability with TMS-evoked muscle responses [12]. They stimulated 10 to 19 subjects with different durations of DC stimulation and measured MEPs of the right *abductor digiti minimi* when applying TMS during and after stimulation. The stimulation times varied from 4 s up to 5 minutes and intensities were between 0.2 mA and 1 mA.

Nitche and Paulus found differences in MEP sizes after 5 minute DC-stimulation between anodal and cathodal stimulation directions. The amplitudes were 40% higher than baseline after anodal stimulation and decreased 30% in comparison to

baseline after cathodal stimulation. No significant difference during the stimulation was found. [12]

In this thesis, simultaneous tDCS and TMS or simultaneous tACS and TMS were applied and resulting MEPs were recorded. The goal was to see the difference among positive, negative, and zero-current conditions. The hypothesis is that when stimulating close to the threshold intensity that barely gives MEP responses, adding the positive current, parallel to the TMS-induced electric field, would increase the number and amplitude of the responses. Similarly, the negative current, anti-parallel to the TMS-induced electric field, would reduce the strength and number of the measured responses. Furthermore, when using TMS slightly above and below the motor-threshold, we tested whether adding the electric current would compensate the intensity change of TMS or enhance or inhibit the total stimulation intensity leading to sub- or supra-threshold effects. The hypothesis is visualized in Fig. 6. If the hypothesis was proven by the results, it would suggest that the current understanding about the mechanisms of transcranial brain stimulation methods is correct.

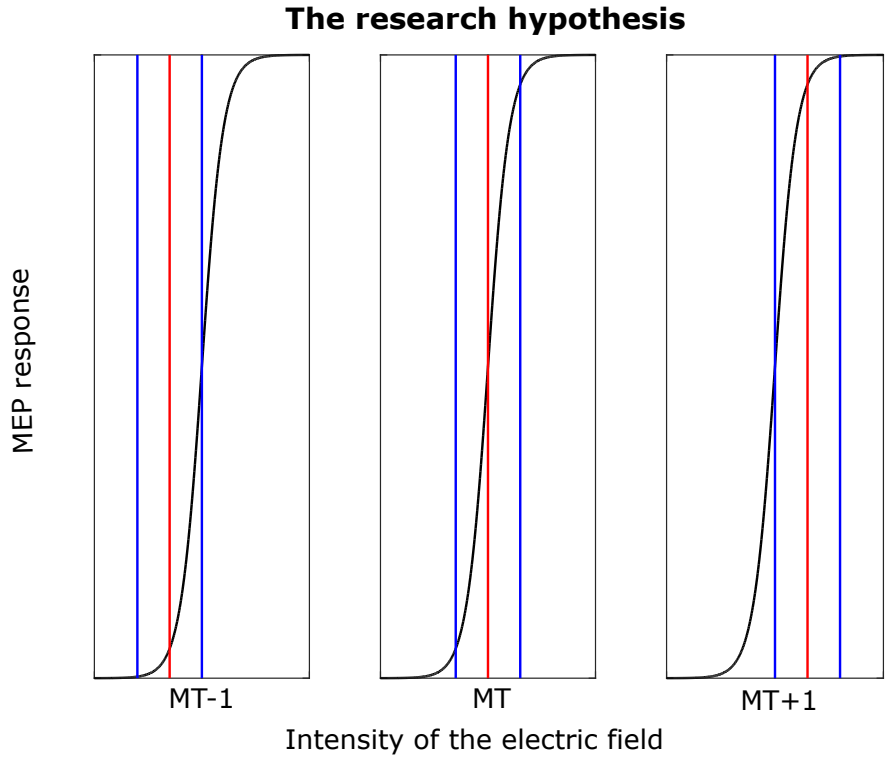

Figure 6: The hypothesis of the stimulation results. This is a simplified model of the expected outcome of the combined TMS-tES. The black curve describes the MEP response curves as a function of the stimulation intensity. The red lines are the TMS-stimulation intensities and the blue lines describe the net stimulation intensity after the positive or negative tES was added.

3 Methods

3.1 Subjects

Data were gathered from 5 healthy voluntary subjects: 4 males and 1 female. The subjects gave their written consent. Four of the subjects were right-handed and one was left-handed. The subjects were of age between 23 and 27 years. The measurements were approved by the Ethics Committee of Helsinki University Central Hospital and they followed the principles of the Declaration of Helsinki.

Table 1: Stimulation parameters of all 5 subjects. The stimulation target refers to the cortical representation of either *abductor pollicis brevis* muscle(ABP) or *first dorsal interosseus* muscle (FDI) and the hemisphere was chosen to correspond the dominating hand of the subject.

Subject	Stimulation target	Hand	Coil type	Current form	Number of stimulations	Gender
Subject1	FDI	right	monophasic	DC	900	female
Subject2	FDI	right	monophasic	DC	900	male
Subject3	FDI	left	monophasic	DC	900	male
Subject4	ABP	right	monophasic	AC	600	male
Subject5	ABP	right	monophasic	AC	600	male

3.2 Experimental paradigms

We designed two different set-ups for the experiments. Two subjects underwent a study with the TMS given simultanously with tACS, and three subjects had combined tDCS–TMS stimulations.

For all the subjects, the measurements were performed with a Nexstim eXimia system with a magnetic stimulator module, a navigated TMS (nTMS) tracking unit and an EMG device. TES was delivered with a neuroConn DC-stimulator. The coil used for TMS-stimulations was the Nexstim's monophasic figure-of-eight coil with the loop diameter of 70 mm. The inter stimulus interval was randomized between two and three seconds. T1-weighted magnetic resonance images had been taken of the subjects before the experiments and uploaded to the system for nTMS. Each subject used earplugs to soften the coil click. The electrical stimulation was given with with 15 cm^2 rubber electrodes. The rubber electrodes, covered with sponges that were moist of saline solution to give a better connection, were placed on the scalp with rubber bands. The DC-stimulator monitored the impedance to detect insufficient skin-electrode contact. The EMG signal was measured from either

abductor pollicis brevis (ABP) or from *first dorsal interosseus* (FDI) muscle of the dominating hand. The first electrode was attached to the target muscle and the other to an adjacent tendon (belly-tendon montage). The reference electrode was placed behind the hand. The sampling frequency of the EMG was 3 kHz.

From all subjects, the stimulation hot spot for the target muscle was identified and personal motor thresholds were defined. To find the right representation area of the muscle, a cortical areawas chosed based on anatomical landmarks and stimulated with relatively high intensity. The stimulation point that gave the highest muscle response was then chosen as the hot spot. Motor-threshold intensity was defined to be the lowest intensity that gave at least 5 out of 10 MEP-responses of at least 50 μV. The electrical stimulation electrode layout was chosen based on the stimulation hotspot. The electrodes were placed to be 15 cm from each other measured from the center of the sponge so that TMS coil would still be able to fit between the sponges without disturbance. The sponge places were chosen to be on the opposite sides of TMS hot spot so that electric field would be approximately parallel to the TMS stimulation direction to get the tES generated electric field to be as large as possible at the hot spot and to the parallel direction with TMS-induced electric field. The electrodes were attached with rubber bands as well as possible. An example set-up is shown in Fig 7.

In the tDCS–TMS measurements, subjects were given 9 different sets of 50 TMS pulses. Each set had a different combination of TMS and tES intensities and the sets were performed in random order. The location of the TMS coil was kept constant with the help of neuronavigation. Each of the conditions was repeated twice so that, in total, 100 stimulation per condition was achieved. The conditions varied in TMS stimulation intensity (MT, MT + 1 % and MT - 1 % of the maximal stimulator output) and in tDCS current amplitude (no current and 2 mV to anodal (positive) and cathodal (negative) directions).

Similar conditioning was done with tACS–TMS stimulations, but instead of using different tDCS conditions tACS was used. tACS was set to vary between -1.5 mA and 1.5 mA with the frequency of 1Hz. Also here, the TMS intensities were set to vary above and below the MT as with tDCS. 200 TMS pulses per TMS intensity were delivered. A pair of EMG-electrodes was used to record the tACS-induced electric field from the neck to get the information of the phase at stimulation time.

3.3 Data analysis

Offline analysis was performed with MATLAB (The Mathworks, Inc., Natick, Massachusetts, USA). First, the data were visually inspected: epochs containing increased EMG baseline activity showing contraction of the target muscle were removed. The first stimulus of each stimulation set is known to have an increased response [46] so they were removed. After removing bad epochs, all the remaining trials were visually inspected to identify MEPs. An EMG deflection was identified as a MEP if it started 20–24 ms after the pulse, had a characteristic bipolar shape and an amplitude clearly above the background noise level. Peak-to-peak values of MEPs were measured manually from the MATLAB figures.

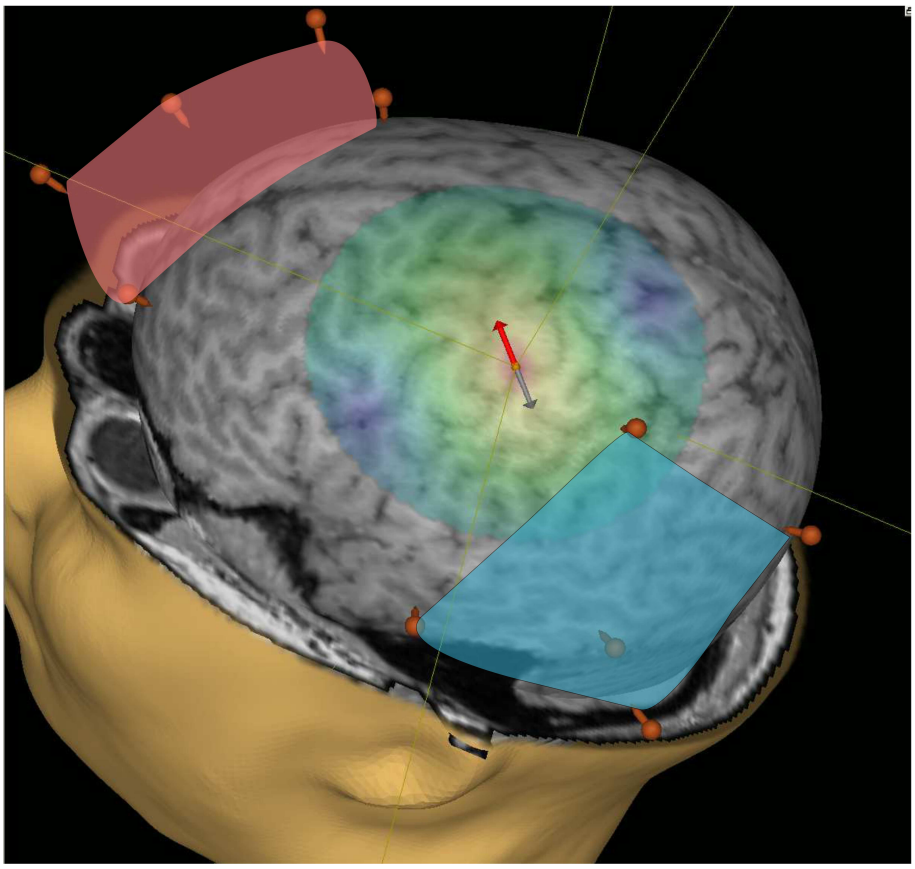

Figure 7: The stimulation set-up. The arrow shows the direction of the TMS-induced electric field. The stimulation point corresponding to the stimulating muscle is in the middle of the arrow. Red and blue areas represent the tES stimulation sponge positions that were chosen based on the stimulation hot spot.

3.4 The tDCS data analysis

With tDCS data, we looked at the probabilities of MEP occurring. For this MEP likelihood $p(\text{MEP})$ was estimated for each of the nine different parameter combinations:

$$p(\text{MEP}) = \frac{N_{\text{MEP}}}{N_{\text{trials}}}\,, \tag{4}$$

where N_{MEP} is the number of observed MEPs and N_{trials} the number of accepted epochs of the same condition.

Also the averages of the peak-to-peak MEP amplitudes over epochs, containing a MEP, were calculated for each condition:

$$\bar{V}_{\text{MEP}} = \frac{1}{N_{\text{MEP}}}\Sigma_{i=1}^{n} V_{\text{MEP}}^{i}\,, \tag{5}$$

where V_{MEP}^{i} is the peak-to-peak amplitude of i:th MEP. And the standard error of the mean (SEM) is calculated by:

$$\mathrm{SEM} = \frac{\sigma_{\mathrm{MEP}}}{\sqrt{N_{\mathrm{MEP}}}}\,, \tag{6}$$

where σ_{MEP} is the standard deviation of the measured amplitudes:

$$\sigma_{\mathrm{MEP}} = \sqrt{\frac{1}{N_{\mathrm{MEP}}-1}\Sigma_{i=1}^{n}(V_{\mathrm{MEP}}^{i} - \bar{V}_{\mathrm{MEP}})^2}\,. \tag{7}$$

3.5 The tACS data analysis

In tACS data, the current parameter varied continuously from -1.5 to 1.5 mA. The tACS data were processed to phase–response data points, where the phase is the phase of the tACS sine wave at the trigger time point. Sine-wave was fitted to the tACS wave measured with EMG electrodes with MATLAB in-built fit-function.

One measure used to evaluate the effect of the current in eliciting MEPs was the moving average (MA) of MEP amplitudes over the phase of tACS:

$$MA(x) = \frac{\Sigma_{i=1}^{n} f(\phi_{\mathrm{MEP}}^{i}) V_{\mathrm{MEP}}^{i}}{f(\phi_{\mathrm{MEP}}^{i})}\,, \tag{8}$$

where ϕ_{MEP}^{i} is the tACS phase at the time of an occurred MEP and V_{MEP}^{i} its corresponding peak-to-peak amplitude. $f(x)$ is the value of density function of normal distribution that was used as the weighting function:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}\,. \tag{9}$$

Here σ is the standard deviation μ is the mean of the distribution and thus the calculation point of the moving average. The standard deviation of 0.5 out of 2π was used.

Same kind of measure was also calculated for the likelihood of MEP occurring:

$$p(\mathrm{MEP}) = \frac{\Sigma_{i=1}^{n} f(\phi_{\mathrm{MEP}}^{i})}{\Sigma_{i=1}^{n} f(\phi_i)}\,, \tag{10}$$

where the ϕ_i is the phase of each epoch.

3.6 Statistical testing

The idea of statistical testing is to test if with some probability it can be said that the hypothesis was right. For example, it can be tested if two sets of measurements come from the same distribution or if there exists a statistical difference between the sets. Another example is to test if the two parameter of the dataset are correlated. Here we wanted to test if there is a difference between the negative and positive current directions by using two statistical tests. In the first study, we tested the two current directions of tDCS against each other with a permutation test. The other

test measured correlation between tACS current strength and the calculated MEP likelihood. The two statistical methods are introduced below.

3.6.1 Permutation test

Permutation test is a good way of testing data with uncommon underlying probability distributions. Using parametric statistical tests to MEP data is not a valid option, because the tests make assumptions on the data distributions tested. Permutation test offers, however, an easy, non-parametric test for data with complicated distributions.

The permutation test works in three phases [47]:

1. Choose the null hypothesis and calculate appropriate measure F that describes the data. F is the test statistic that will be tested.

2. Permute the data so that you pick randomly data from the existing data to the test groups and calculate the same measure F^{π} from this new data set. Repeat this multiple times.

3. Calculate a p-value of how often the new datasets fills the null hypothesis:

$$P = \frac{(No.\, of\, F^{\pi} \geq F)}{(Total\, no.\, of\, F^{\pi})}\,. \tag{11}$$

Here we used the permutation test to compare the dataset distributions of negative and positive current with all three different TMS intensities for each subject. As the comparable measure the TMS likelihood was chosen and calculated for each condition. The null hypothesis was that the MEP likelihood with both tES currents was the same:

$$H_0 : F = p(MEP_{2v}) - p(MEP_{-2v}) = 0\,. \tag{12}$$

Permutations were repeated 100 000 times to get accuracy to the results. To get the p-value the original F-value was compared to the new F^{π}-values.

In statistical testing, when enough statistical comparison are made, it is already probable that some of them are by chance wrong. Already with tDCS data we tested, in total, 9 cases. This is why the Bonferroni correction was used. In the Bonferroni correction the chosen confidence level value is divided by the number of tests made:

$$p <= \frac{\alpha}{n}\,, \tag{13}$$

where p is p-value of the test, α is the confidence level and n is the number of tests performed. Here we used the confidence level of 0.05 with two tailed test.

3.6.2 Correlation test

For the tACS, we had two continuous distributions to compare: the strength of the tACS electric current and the likelihood of MEPs occurring as a function of the tACS

phase. We wanted to see if there is a correlation between the two variables. This can be done using the non-correlation test. The MEP likelihoods can not be modelled as random variables. For this reason, we chose Spearman's test for non-correlation and not Pearson's correlation that assumes distributions to follow normal distribution. [48] The Spearman's test works as following. Order datapoints according to their values for both directions:

$$R(x_i) = \text{Rank of observation } x_i \text{ of pair } i$$
$$R(y_i) = \text{Rank of observation } y_i \text{ of pair } i$$

and the difference is defined to be:

$$D_i = R(x_i) - R(y_i),\ i = 1,\ 2,\ ...,\ n.$$

The Speraman's rho, is defined as:

$$\rho_S = 1 - \frac{6\Sigma_{i=1}^{n} D_i^2}{n^3 - n} \tag{14}$$

and the test statistics through that:

$$z = \sqrt{n-2}\frac{\rho_S}{\sqrt{1-\rho_s^2}}\,. \tag{15}$$

The zero hypothesis H_0 is here defined to be $H_0 : \mathrm{Cor}(x, y) = 0$. If this is true the test statistic z follows the standardized normal distribution $N(0, 1)$. The large absolute values of z indicate that zero hypothesis is wrong and there is correlation. The confidence level used here is again two tailed 0.05, with Bonferroni correlation.

4 Results

The data from the measurements were preprocessed as described in section 3. The sample raw data resulting from the tACS measurements is shown in Fig. 8 A and B. Fig. 8 C shows an example of preprocessed MEP-values. The B and C would look similar for the tDCS data. The sine-wave fits to the measured tACS wave perfectly and clears off the ripples. The MEP-data seen in the sample (Fig. 8 C) is variable: most of the MEP-amplitudes are below 200 μV, but few have an amplitude of over 800 μV.

The motor thresholds of each subject are listed in Tab. 2. The average max electric field values during stimulation, as the estimated by the TMS navigation system, are also given in Tab. 2. The average max electric field values seem to vary a lot and do not always clearly grow with increasing stimulation intensity as stimulator output.

Table 2: Stimulation intensities used in the measurements. The estimated motor threshold values as the percentual of the maximal machine output and as V/m values for each subject are shown. The averages of the estimations of the neuronavigation system of the maximal electric field during each stimulation are calculated for each condition and subject. The - and + mark the current direction.

Subject	MT-values			Mean max. EF [V/m]		
	% of output	[V/m]		(MT - 1)	(MT)	(MT + 1)
Subject1	50	62	-	63	62	62
				61	63	64
			+	62	63	62
Subject2	65	92	-	93	94	96
				94	95	96
			+	93	93	96
Subject3	59	87	-	90	89	97
				86	89	92
			+	90	89	92
Subject4	44	68		67	70	70
Subject5	56	84		83	87	86

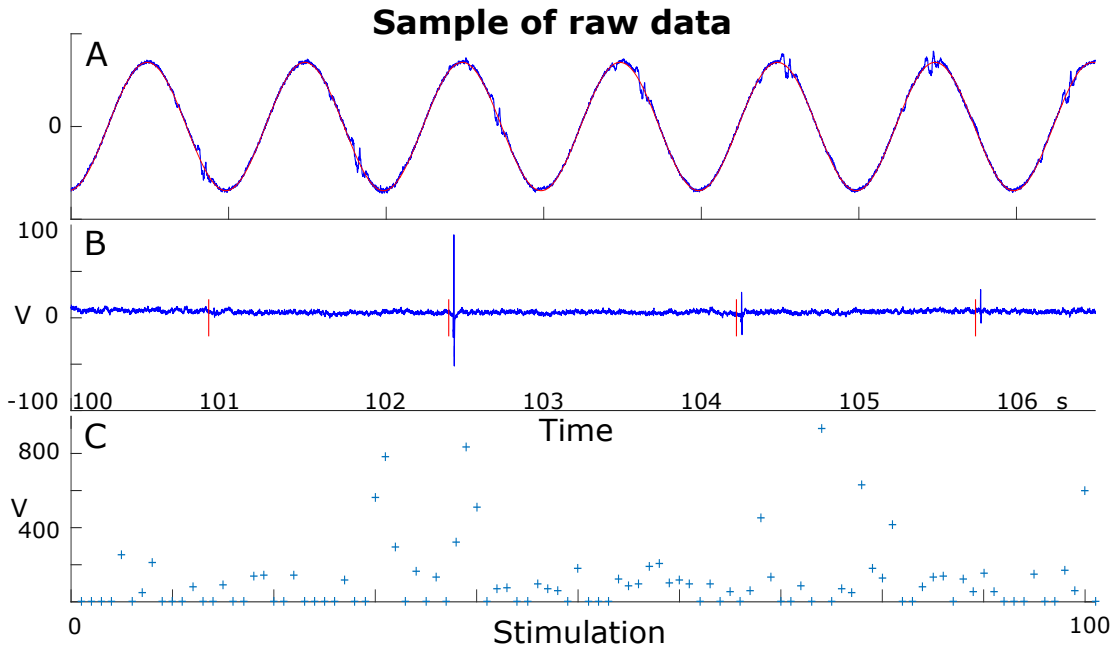

Figure 8: Sample raw data. The measured tACS current (A) and raw EMG data (B), with the trigger time points with red, mapped against time. The C shows the sample of 100 stimulus MEP responses.

4.1 TDCS effects are not clear

TDCS current does not seem to have a clear effect on MEP responses (Fig. 9 and Fig. 10). Fig. 9 represents the likelihoods of MEPs occurring after TMS stimulation, for all 9 conditions and all subjects and Fig. 10 represents the mean amplitudes for all 9 conditions and all subjects. There is no clear trend visible between the negative current direction, no current and positive current direction in either of the cases.

The difference between the two current directions was assumed to have the greatest difference, so test statistics were calculated for MEP-likelihoods in all three conditions of different intensities and to all subjects using permutation tests. Many of the current pairs resulted in statistical significance between the values $p(<0.05)$, but the direction of the significance changed. Results can be seen in Table 3.

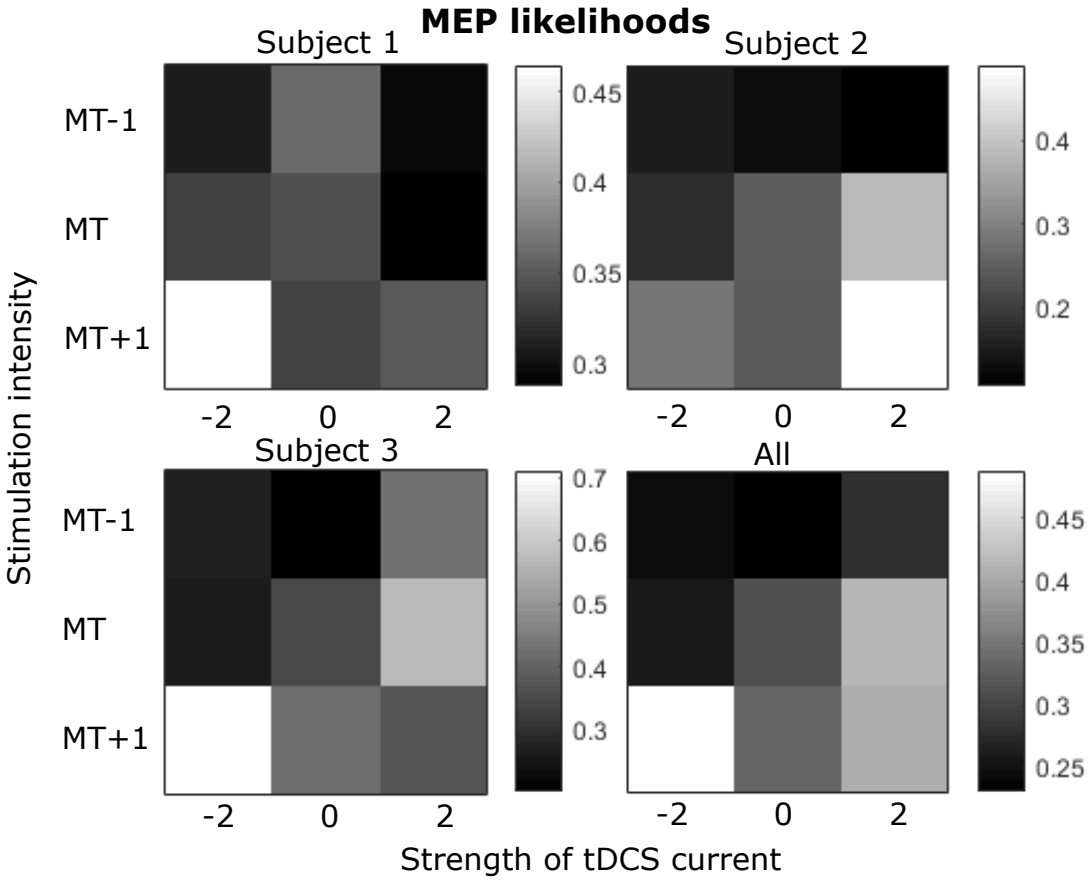

Figure 9: The MEP-likelihoods in each tDCS–TMS condition. MEP-likelihoods for all 9 conditions for all 3 subjects and for summation over all subjects have been calculated. The current value changes on the x-axis and the intensity of the machine as function of the motor threshold is on the y-axis.

4.2 TACS has little to no affect on MEP responses

The moving average values of MEP amplitudes, showed in Fig. 11, do not seem to be depending on the current strength or vary along the phase of the current. The Fig. 12 and the Fig. 13 show the MEP likelihood variation along the current phase and strength, respectively. The correlation between the current strength and MEP likelihood values were tested with the Spearman's test. In two conditions for subject 4 and in one condition of subject 5, there were significant correlations between the two values, but once again the correlation direction varied. The p-values can be seen in table 3.

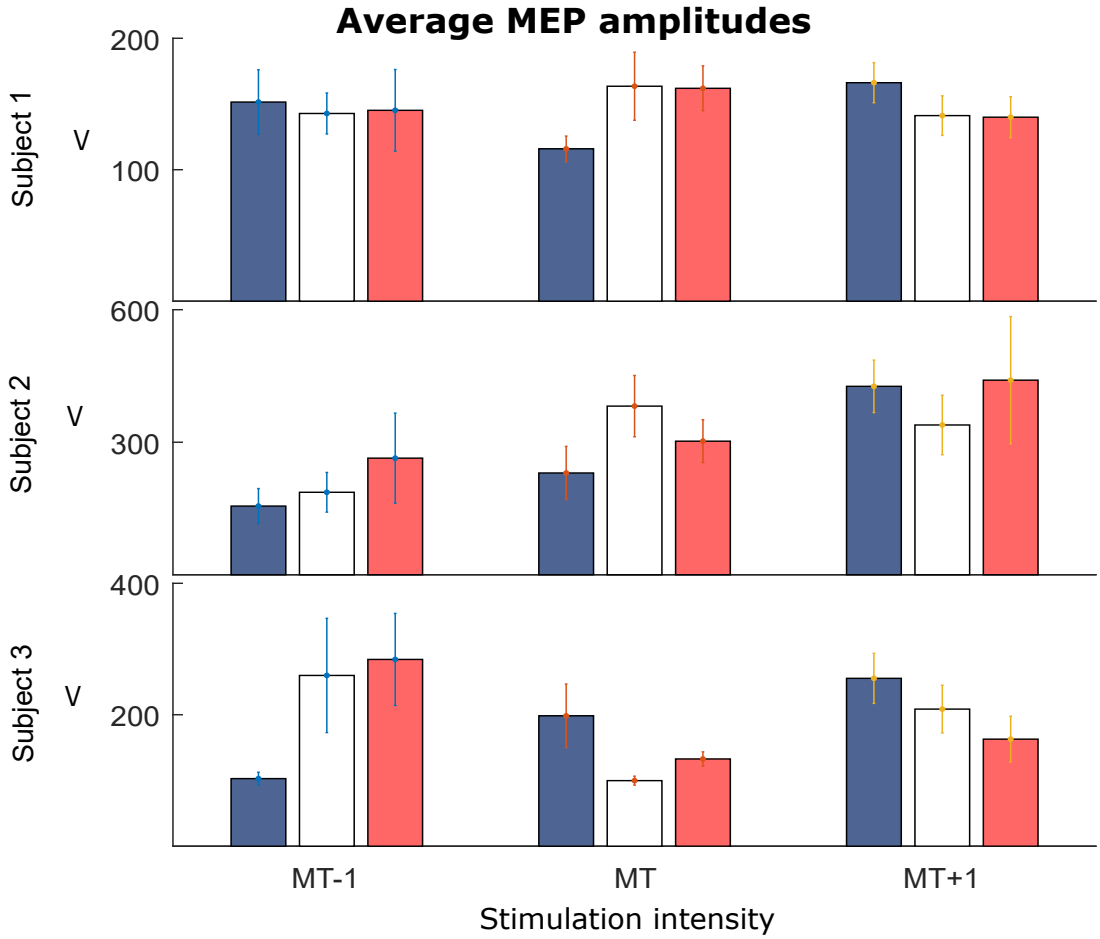

Figure 10: The MEP amplitudes per condition. MEP amplitudes for all 9 conditions for all 3 subjects were calculated. The blue bars stand for the negative current direction, white bars for no current, and red bars stand for the positive current direction.

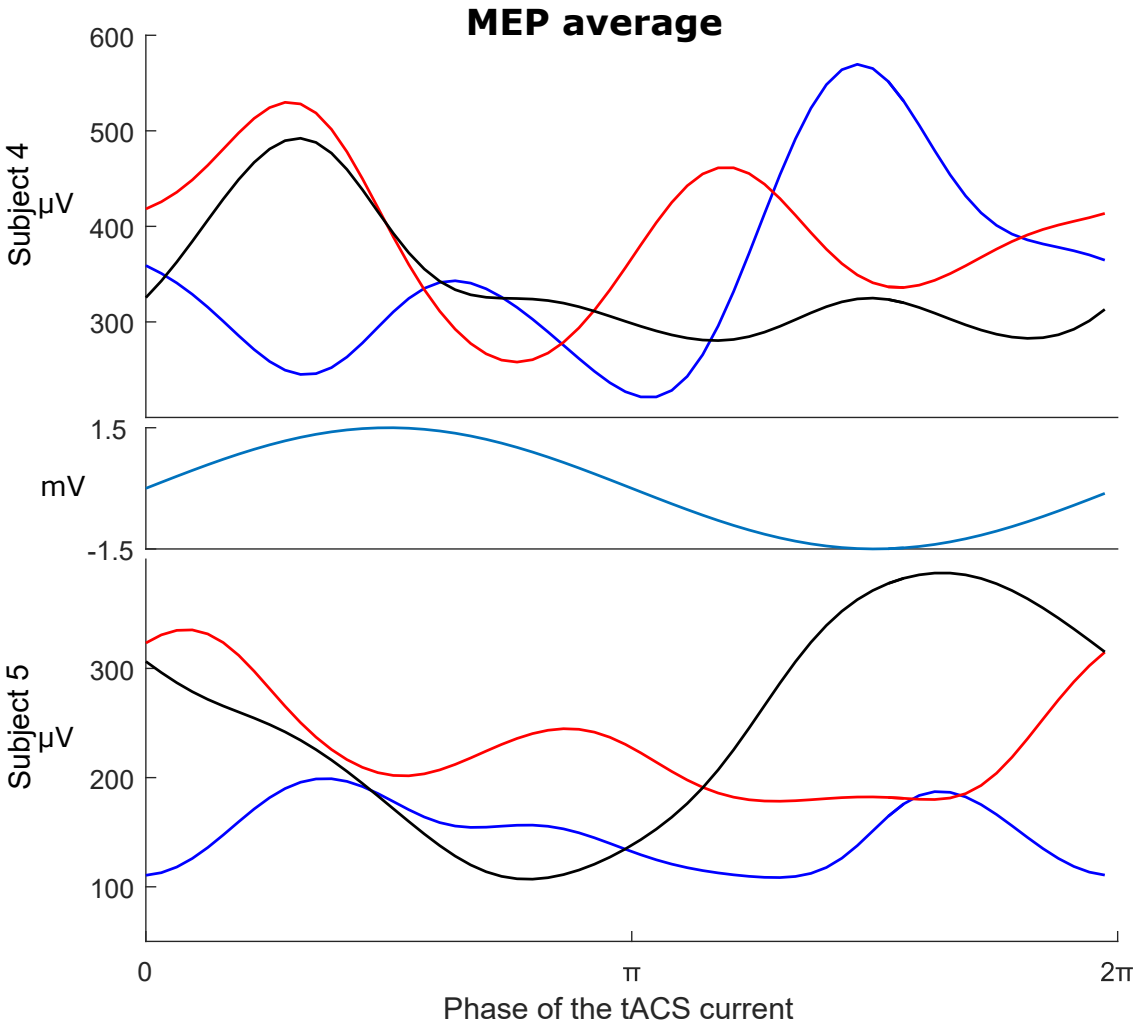

Figure 11: The moving averages of MEP amplitudes for the two subjects. Blue lines stand for intensity MT-1, black ones for MT and the red lines stand for MT+1 intensity. On the x-axis, there is the phase of the tACS. The blue curve in the middle is the tACS current strength at that phase.

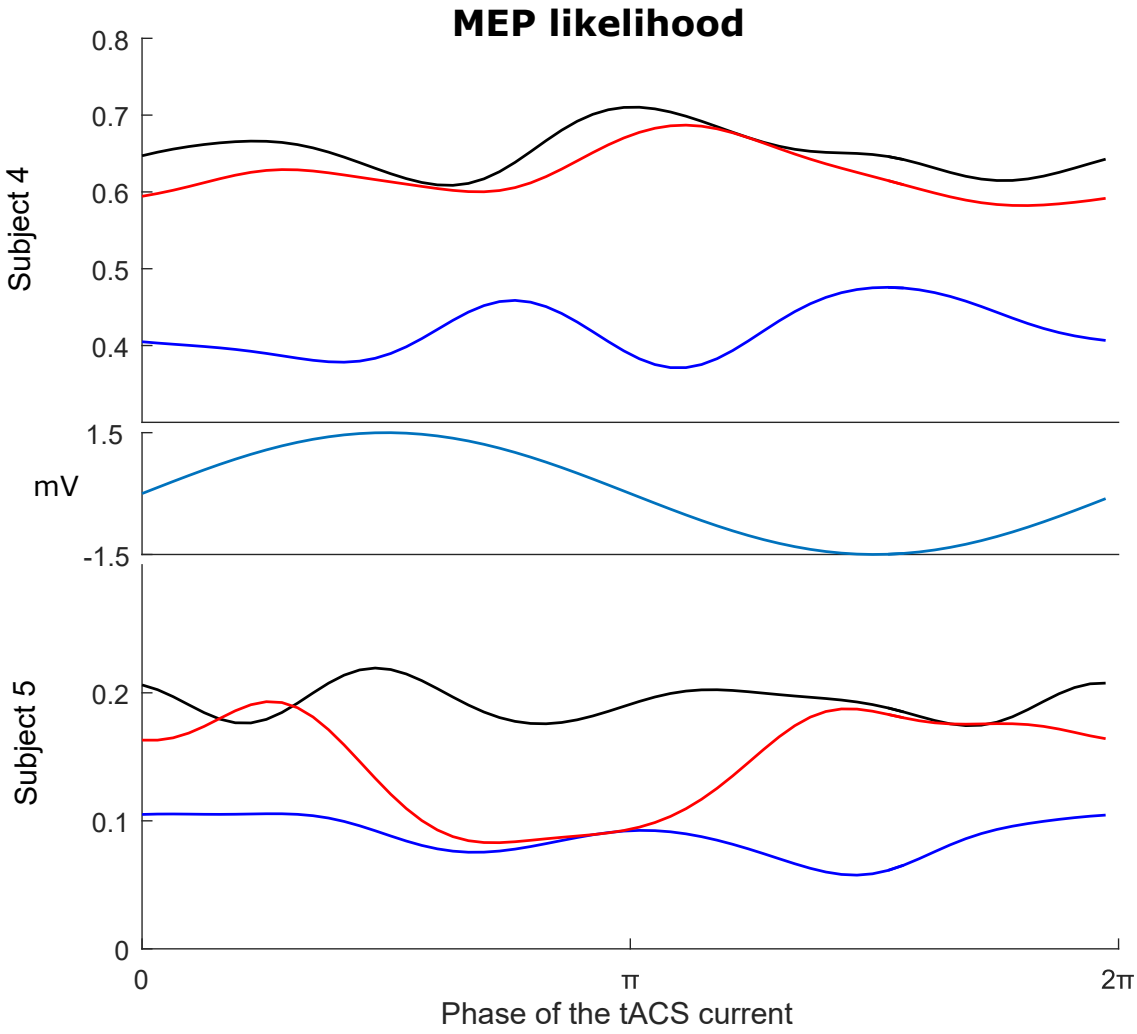

Figure 12: The moving averages of MEP likelihoods for the two subjects. Blue lines stand for intensity MT-1, black ones for MT and the red lines stand for MT+1 intensity. On the x-axis, there is the phase of the tACS. The blue curve in the middle is the tACS current strength at that phase.

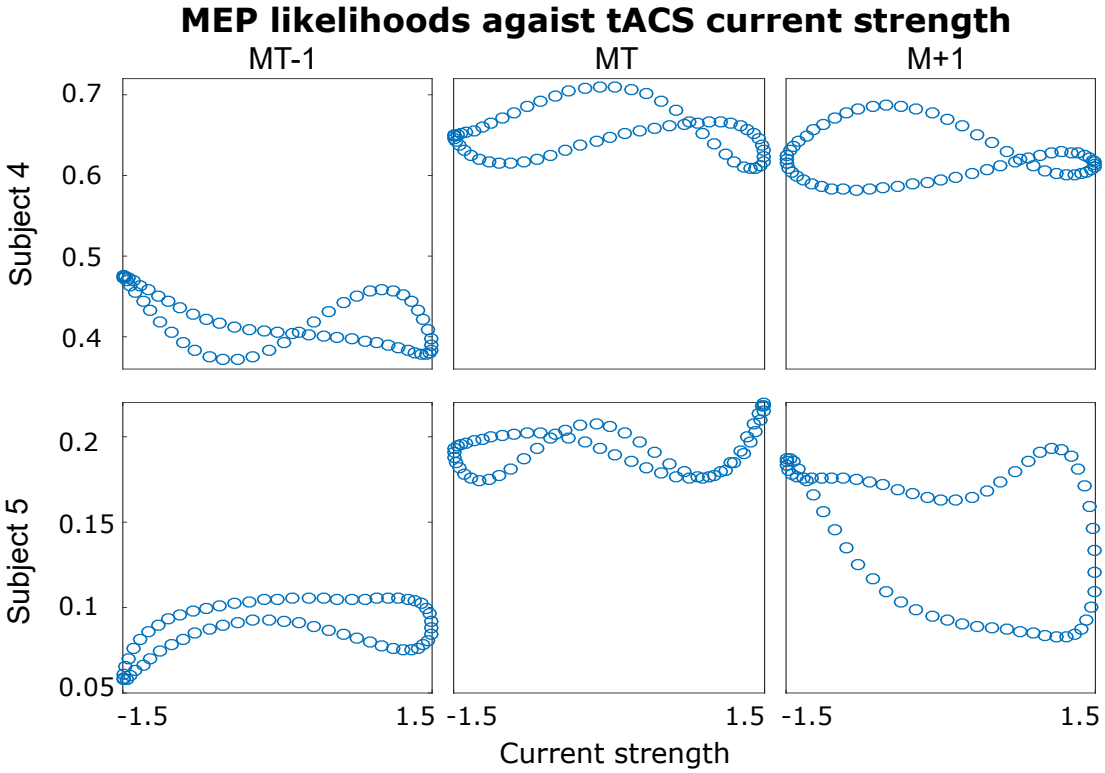

Figure 13: The moving MEP likelihoods plotted against the tACS current strength. At many cases, the stimulation outcome is similar to both current directions with the same current strength.

Table 3: Statistical p-values for the difference between the negative and positive current directions for the first three subjects. For subjects four and five p-values counted for non-correlation. The values with statistical significance are marked with a star. The '-mark describes p-values of values greater than the mean of the normal distribution.

Subject	MT - 1	MT	MT + 1
Subject1	'0.462	'0.290	'0.066
Subject2	'0.229	0.000*	0.001*
Subject3	0.007	0.000*	'0.000*
Subject4	'0.000*	'0.033	0.000*
Subject5	0.000*	0.299	0.411

5 Discussion

The results in this work did not confirm the current understanding of the mechanisms of transcranial brain stimulation methods. It is possible that tDCS has only long term effect to the neuronal messaging that takes place due to long term potentiation or long term depression of the synaptic transmission [25]. The longer-term effects of tDCS have been shown to exist already in previous studies [12].

The short-term effect of the tDCS was hypothesized to either enhance or inhibit the effect of the TMS, causing more or less neurons to be activated. If the tES de- or hyperpolarizes sufficiently the same axons as TMS, it should have been visible in the results.

The results show statistically significant differences between the negative and positive tES currents. However, the results are not consistent; sometimes the positive current seemed to enhance the stimulation, while occasionally the outcome was the opposite. The ambiguous results may be may be due to natural random fluctuations in the cortical activity or they may be caused by other parameters that were not controlled well enough. It is also possible, that the significant changes were caused by tES, but the working principle of tES is much more complex than we have assumed.

5.1 Possible reasons for the ambiguous results

MEPs are a highly variable measure to analyse [37]. For example, the brain state and the tiredness of the subject can vary during the measurements. To avoid the systematic effects of changes in the brain state, the measurements were performed in small stimulus sets in a random order of conditions. The randomized order might not, however, remove the effect perfectly. Occationally, the subject can think of moving his or her finger that is known to affect MEP responses [49]. It is well known, that the MEP measures even with constant stimulus parameters can vary considerably.

With the fixed coil location set on in the Nexstim TMS-system, the location can vary up to 2 mm in targeting and 2 degrees in the tilting and orientation accuracy [21]. The coil location change might result in a significant change of the TMS-induced electric field.

The estimation of the max electric field given by the Nexstim navigation system does not seem to follow the raise of intensity as % of stimulator maximal output (Tab. 2). The coil-movement elicited changes in the TMS-induced electric field might be one explaining factor of the surprising results. For example, in the case of the intensity of MT + 1 of Subject 3, the difference of the average maximal electric field of TMS is 5 V/m between the negative and positive tDCS direction.

As we can see there is a visible trend, that TMS stimulation intensity has an impact on the MEP-likelihood as can be seen from the Fig. 9 and Fig. 12. The effect is not as clearly visible in the case of MEP amplitudes (Fig. 10 and Fig. 11). The raise of the likelihoods when rising the TMS stimulation intensity was expected, because higher intensity induces higher electric field that depolarizes the neurons

more resulting in an increased number of action potentials.

The simulated strength of the electric field in the cortex resulting from the tES of 2 mA was only 0.22V/m [22]. In comparison to the electric field induced by TMS, it is very small. Also the number of the stimulations per condition in each subject might have been too small to bring out the effect of tES. Furthermore, the tES-induced electric field seems even smaller than the variation of estimated maximal electric fields of TMS stimulation, possibly masking the tES-elicited changes. With large enough a number of epochs the fluctuations in the effect of TMS-induced electric fields would also be decreased as the strength of the maximal electric field for each TMS intensity would probably approximately follow the normal distribution.

The tES sponge locations could have been more optimal. Here, the sponge locations were chosen with our best knowledge based on earlier computational results of the tES current flow in the brain. Fixing the tES sponges with rubber bands was difficult and as a result, the sponges were only roughly at the spots we had decided. To improve the fixing of the tES electrodes, an electrode paste that glues the electrodes to the scalp could be used instead of rubber bands.

5.2 Future prospects

Based on the obtained results, we cannot exclude the possibility that tES works as hypothesized. However, to show this, more measurements need to be performed. In these measurements, one should be really careful with controlling the electric fields of TMS stimulation. When the effect of the electric field strength of TMS could be decreased remarkably it might be possible to see the effect of the tES-induced electric field.

To get the optimal tES-induced fields to the stimulation point, tES sponge locations could be modelled for each subject separately, utilizing the anatomical information of the head of the subject. In addition the sponges should be fixed to the scalp with better methods. Also higher tES stimulation intensities should be used if possible.

6 Conclusion

Both tES and TMS are methods that are proven to have an effect on the neuronal messaging, but their working methods are not certain. The purpose of this work was to better understand how they work by combining these two different methods. We were expecting to see a difference between the positive and the negative electric field caused by tES stimulation in the EMG responses when TMS stimulation was given simultaneously. The difference between the two current directions was not clearly visible from the results of this study. It is still unclear if this is because tES has no effect or due to some parameters that should be better controlled during the measurements. It is also possible that the effect of tES is so small that it will always be difficult to measure reliably. To be certain of the cortical effects of these methods, more research must be done.

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